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TESI DI SPECIALIZZAZIONE

PROGNOSTIC VALUE OF HER2-LOW STATUS IN BREAST CANCER: A SYSTEMATIC REVIEW AND META-ANALYSIS

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Summary

Abstract

Background: Human epidermal growth factor receptor 2 (HER2)-low expression in breast cancer has been recently identified as a new therapeutic target. However, it is unclear if HER2-low status has an independent impact on prognosis.

Materials and methods: A systematic literature research was carried out to identify studies comparing survival outcomes of patients affected by HER2-low versus HER2-zero breast cancer. Using random-effects models, pooled hazard ratios (HRs) and odds ratios (ORs) with 95% confidence intervals (CIs) were calculated for progression-free survival (PFS) and overall survival (OS) in the metastatic setting as well as disease-free survival (DFS), OS and pathological complete response (pCR) in the early setting. Subgroup analyses by hormone receptor (HoR) status were carried out. The study protocol is registered on PROSPERO (n.CRD42023390777).

Results: Among 1916 identified records, 42 studies including 1 797 175 patients were eligible. In the early setting, HER2-low status was associated with significant improved DFS (HR 0.86, 95% CI 0.79-0.92, P < 0.001) and OS (HR 0.90, 95% CI 0.85-0.95, P < 0.001) when compared to HER2-zero status. Improved OS was observed for both HoR-positive and HoR-negative HER2-low populations, while DFS improvement was observed only in the HoR-positive subgroup. HER2-low status was significantly associated with a lower rate of pCR as compared to HER2-zero status both in the overall population (OR 0.74, 95% CI 0.62-0.88, P=0.001) and in the HoR-positive subgroup (OR 0.77, 95% CI 0.65-0.90, P=0.001). In the metastatic setting,

patients with HER2-low breast cancers showed better OS when compared with those with HER2-zero tumours in the overall population (HR 0.94, 95% CI 0.89-0.98, P=0.008), regardless of HoR status. No significant PFS differences were found.

Conclusions: Compared with HER2-zero status, HER2-low status appears to be associated with a slightly increased OS both in the advanced and early settings, regardless of HoR expression. In the early setting, HER2-low tumours seem to be associated to lower pCR rates, especially if HoR-positive.

Introduction

Breast cancer is one of the most common malignancies worldwide [1]. It is traditionally classified into different subtypes, according to hormone receptor (HoR) expression and human epidermal growth factor receptor 2 (HER2) status: luminal-like (HoR-positive/HER2-negative), triple negative (HoR negative/HER2-negative) and HER2 positive (HoR-positive or negative), partially resembling the molecular luminal A, luminal B, HER2-enriched and basal-like subtypes [2,3]. According to the American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) guidelines, HER2 positivity is defined by an immunohistochemical (IHC) score of 3+ or 2+ with in situ hybridization (ISH) amplification. An IHC score of 0, 1+ and 2+ without ISH amplification would define a tumour as HER2-negative [4]. In recent years, a new concept has emerged in the breast cancer scenario: tumours characterized by an IHC score of 1+ and 2+ without ISH amplification are defined as HER2-low [5,6]. These tumours, previously categorized as HER2-negative, have been recently identified as a therapeutic target for new HER2-targeting antibody-drug conjugates (ADCs), like trastuzumab deruxtecan (T-DXd). T-DXd was compared to a physician's choice chemotherapy in HER2-low metastatic breast cancer patients treated with one or two previous lines of chemotherapy within the DESTINYBreast04 phase III trial. The study showed notable improvements in progression-free survival (PFS) and overall survival (OS) with T-DXd in the overall population enrolled, as well as in the HoR-positive and triple-negative subcohorts, separately [7]. Based on these results, T-DXd was recently approved by the Food and Drug

Administration and European Medicines Agency for the treatment of patients with advanced HER2-low breast cancer, representing the first approved treatment indication in this subpopulation [8,9]. Despite its therapeutic implications, it is unclear if HER2- low status has an independent impact on prognosis, both in the metastatic and early settings. Several studies have investigated the prognostic value of HER2-low status with conflicting results [10]. In order to address this controversial topic, we conducted a systematic review and meta-analysis to assess the prognostic role of HER2-low status in breast cancer, both in early and advanced settings and according to HoR status.

Materials and Methods

We conducted a quantitative synthesis of data from studies evaluating the prognostic role of HER2-low status, in the early and advanced settings and according to HoR status.

Search strategy and study identification

We carried out a systematic literature research of PubMed and Cochrane databases with no language or date restriction up to 18 December 2022. We also retrieved abstracts from major international conferences of the past 2 years [American Society of Clinical Oncology (ASCO), European Society of Medical Oncology (ESMO) and ESMO Breast, San Antonio Breast Cancer Symposium (SABCS)] in order to identify potentially eligible unpublished studies. The search strategy was carried out using the keywords ‘breast cancer’, ‘HER2-Low’, ‘ERBB2-low’,

'human epidermal growth factor receptor 2 low', 'low level HER2'. The full search strategy used for each database is presented in the Supplementary Material. The systematic literature research was carried out independently by two authors (CM and FJ) and any discrepancies were solved by discussion with a third author (EA). The present systematic review and meta-analysis was carried out according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [11]. This study is registered in the PROSPERO database (registration number CRD42023390777) and the protocol is available in the PROSPERO website.

Selection criteria and data extraction

To be included in the present meta-analysis, studies had to satisfy the following inclusion criteria: (i) studies including patients diagnosed with invasive breast cancer with any disease stage I-IV; (ii) studies reporting the prognosis of patients with HER2-low breast cancer in comparison to those with HER2-zero breast cancer. If more than one publication on the same dataset was available, data were extracted from the most updated record. Studies meeting one of the following criteria were excluded: (i) insufficient results on the association between HER2-low status and clinical outcomes; (ii) studies reporting on HER2-low status in patients not affected by breast cancer; (iii) studies published in languages other than English. The following variables were extracted from the included studies, when available: author, year of publication, country, median follow-up, type of study, total number of patients, number of patients with HER2-low breast cancer, number of patients with HER2-zero breast cancer,

number of patients with HER2-low/HoR-positive breast cancer, number of patients with HER2-zero/HoR-positive breast cancer, number of patients with HER2-low/HoR-negative breast cancer, number of patients with HER2-zero/HoR-negative breast cancer, type of comparison, disease-free survival (DFS), pathological complete response (pCR) and OS in the early setting for each patients' subgroup, PFS and OS in the metastatic setting for each patients' subgroup.

Study objectives

The primary endpoint of our meta-analysis was to assess the prognostic value of HER2-low status in breast cancer, both in the early and advanced settings. The primary objectives were to evaluate: (i) the association between HER2- low status and pCR rate, DFS and OS in the early setting; (ii) the association between HER2-low status and PFS and OS in the advanced setting. Secondary objectives of our analysis were assessing (i) the association between HER2-low status and pCR rate, DFS and OS in the early setting according to the HoR status and (ii) the association between HER2-low status and PFS and OS in the metastatic setting, according to the HoR status.

Risk of bias assessment

The risk of bias (RoB) for each included study was evaluated by two investigators (CM and GNM). The RoB was assessed using the Quality in Prognosis Studies (QUIPS) tool [12], which includes six distinct domains regarding study participation, study attrition, prognostic factor measurement, outcome measurement, study confounding, statistical analysis and reporting. Through this tool, each study was classified as having a low, moderate or high RoB.

Statistical analysis

We calculated the pooled hazard ratio (HR) comparing patients with HER2-low breast cancer and HER2-zero breast cancer for survival endpoints in the early setting (DFS and OS) and in the metastatic setting (PFS and OS), and the pooled odds ratio (OR) for the pCR endpoint. The random-effects model of Der Simonian and Laird was applied to compute the pooled estimates of HR and OR and their 95% confidence intervals (CIs). This model allowed us to estimate the amount of the variability between studies and accordingly provide suitable standard errors of pooled HR and pCR. We used the random-effects model even if the heterogeneity between studies was low since, when the studies included in a meta-analysis derived from the published literature, the assumption that they all share an identical true effect size and the differences are exclusively due to the sampling error, as required by the fixed-effects model, is too stringent. Nevertheless, when the heterogeneity is low, fixed- and random-effects models provide similar results [13]. When available, HR based on multivariate analysis was used; if not available, we used HR based on univariate analysis. When the OR or HR estimates were not reported but the number of events for each group could be derived, ORs were computed as the odds of events between groups, whereas HRs were estimated using the method reported by Watkins and Bennett [14]. Survival analyses were then repeated by excluding computed HRs and including only the studies reporting the HRs. The Higgins I^2 index was computed to assess the degree of consistency of the results of the studies. Egger's test was used to assess the likelihood of publication bias. To verify if some study strongly influenced the pooled estimates, sensitivity

analyses were carried out, by excluding the studies one at a time and recalculating the pooled estimates. All statistical analyses and forest plot generations were carried out using STATA Software Version 13.1 (StataCorp LP, College Station, TX). Cohorts including merely HoR-positive tumours were only included in the HoR-positive subgroup analysis. Cohorts including exclusively HoR-negative tumours were only included in the HoR-negative subgroup analysis.

Results

A total of 1916 records were identified from databases and conference proceedings by using the above-mentioned research criteria. After duplicate removal and exclusion of non-relevant records, 42 studies were included in the present meta-analysis (Figure 1). Among them, 12 studies included data from patients affected by metastatic breast cancer [15-26], 27 analysed data from patients with early breast cancer [27-53] and 3 studies analysed subjects in both settings [54-56]. A total of 1 797 175 patients were eligible for this analysis, of whom 1 697 079 had early disease (1 118 389 HER2-low and 578 690 HER2-zero) and 100 096 had advanced disease (59 798 HER2-low and 40 298 HER2-zero).

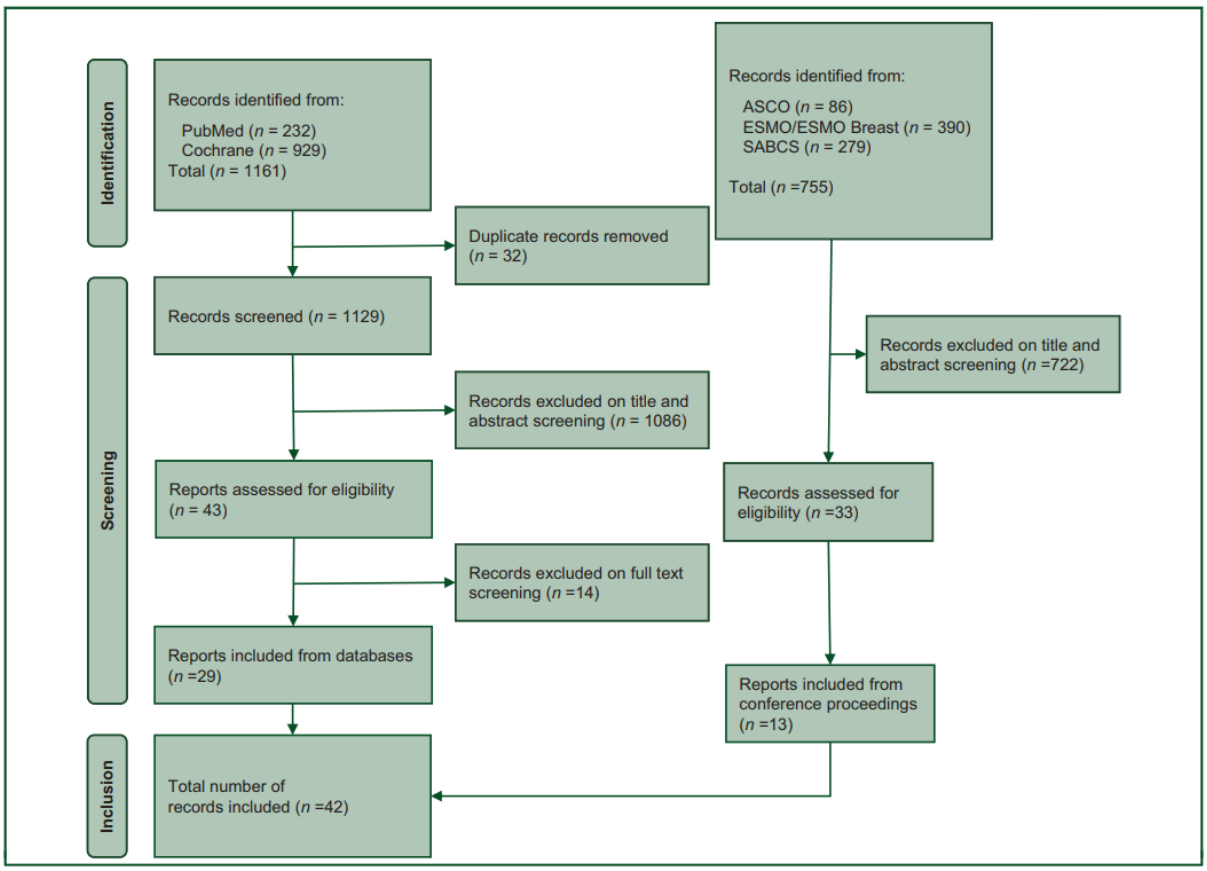


Figure 1. The PRISMA flow chart summarizing the process for the identification of eligible studies. ASCO, American Society of Clinical Oncology; ESMO, European Society for Medical Oncology; PRISMA, Preferred Reporting Items for Systematic Reviews and MetaAnalyses; SABCS, San Antonio Breast Cancer Symposium

Early setting

Pathological complete response

Considering the overall population, 14 studies including 114 754 patients [28,30,31,33,34,38,41,42,46,49,52-55] had available data regarding pCR. A total of 10 675 out of 68 059 (15.6%) patients with HER2-low breast cancer achieved pCR at surgery, compared to 10 593 out of 46 695 (22.6%) patients with HER2-zero breast cancer. A statistically significant difference in terms of pCR in favour of HER2-zero subgroup was found (OR 0.74,

95% CI 0.62-0.88, P=0.001; I^2= 69%; P < 0.001) (Figure 2). The sensitivity analysis provided consistent results with similar OR estimates (Supplementary Table S1). Egger's test P value was 0.024, showing a potential publication bias.

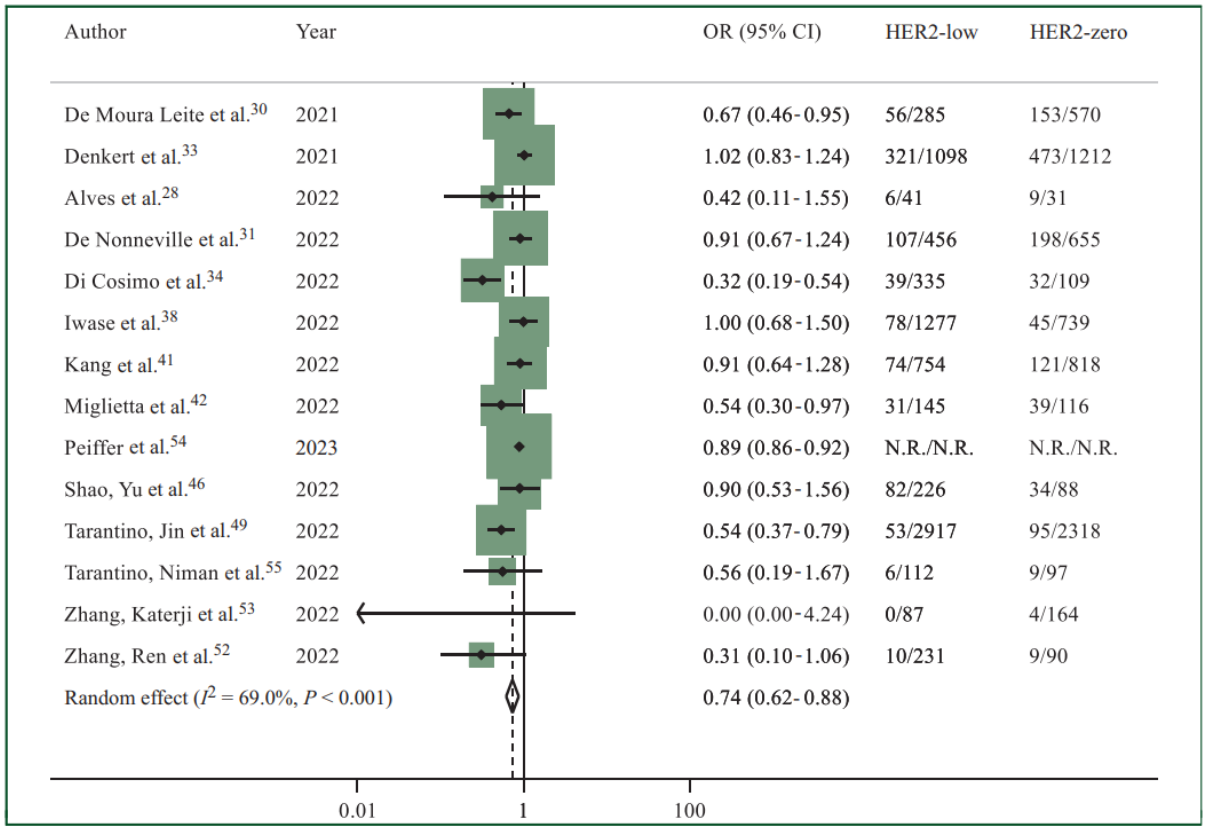


Figure 2. Odds ratio (OR) for pathological complete response after neoadjuvant chemotherapy of HER2-low breast cancers versus HER2-zero breast cancers in the overall population (the size of the squares is proportional to the weight of each study). CI, confidence interval; HER2, human epidermal growth factor receptor 2. Random effect: P=0.001; Egger's test: P=0.024.

In the HoR-positive cohort, pCR data were reported by 13 studies [28,30,31,33,34,36,41,42,46,49,52,54,55]. HER2-low status was significantly associated with a lower rate of pCR (OR 0.77, 95% CI 0.65-0.90, P= 0.001; I^2=17.3%; P= 0.269) (Supplementary Figure S1, sensitivity analysis available in the Supplementary Material and

Supplementary Table S2). In the HoR-negative cohort, pCR data were available for 15 studies [28,30,31,33-35,41,42,46,47,49,51,52,54,55]. No statistically significant difference was found in pCR rates between patients with HER2-low and those with HER2-zero tumours (OR 0.95, 95% CI 0.81-1.11, P= 0.497; I^2=42.5%; P=0.042) (Supplementary Figure S2, sensitivity analysis available in the Supplementary Material and Supplementary Table S3). No significant publication bias was observed for pCR analyses both in HoR-positive and HoR-negative subanalyses (Egger's test: P=0.804 and P=0.513, respectively).

Disease-free survival

Sixteen studies reported DFS results in the overall population [27,28,30,31,33,34,38,41,42,46,48-50,52,55,56]. HER2-low status was significantly associated with longer DFS as compared to HER2-zero status (HR 0.86, 95% CI 0.79-0.92, P < 0.001; I^2= 24.4%; P=0.178) (Figure 3). Consistent results were reported in the sensitivity analysis (Supplementary Table S4). Similar results were observed in the analysis where computed HRs were excluded (data not shown). No publication bias was detected (Egger's test: P=0.212). Among the 20 studies reporting DFS results in the HoR-positive cohort [27,29,30,32-34,36-38,41-46,48,49,50,52,55], HER2-low status was significantly associated with longer DFS as compared to HER2-zero status (HR 0.86, 95% CI 0.80-0.93, P < 0.001; I^2=17.8%; P=0.232) (Supplementary Figure S3). Consistent results were reported in the sensitivity analysis (Supplementary Table S5). No publication bias was found (Egger's test: P=0.357). No statistically significant difference in terms of DFS was found between patients with HER2-low

and those with HER2-zero tumours, analysing 17 studies reporting data from patients with HoR-negative disease (HR 0.90, 95% CI 0.78-1.04, P=0.155; I^2=35.6%; P=0.073) (Supplementary Figure S4) [27,30,33-35,37,39,41,42,44,46-50,52,55]. Egger's test P value was 0.928 showing no RoB. Sensitivity analysis showed a significant difference in favour of HER2-low tumours after the exclusion of the study by Di Cosimo et al. [34] (HR 0.88, 95% CI 0.77-0.99, P=0.038) (Supplementary Table S6).

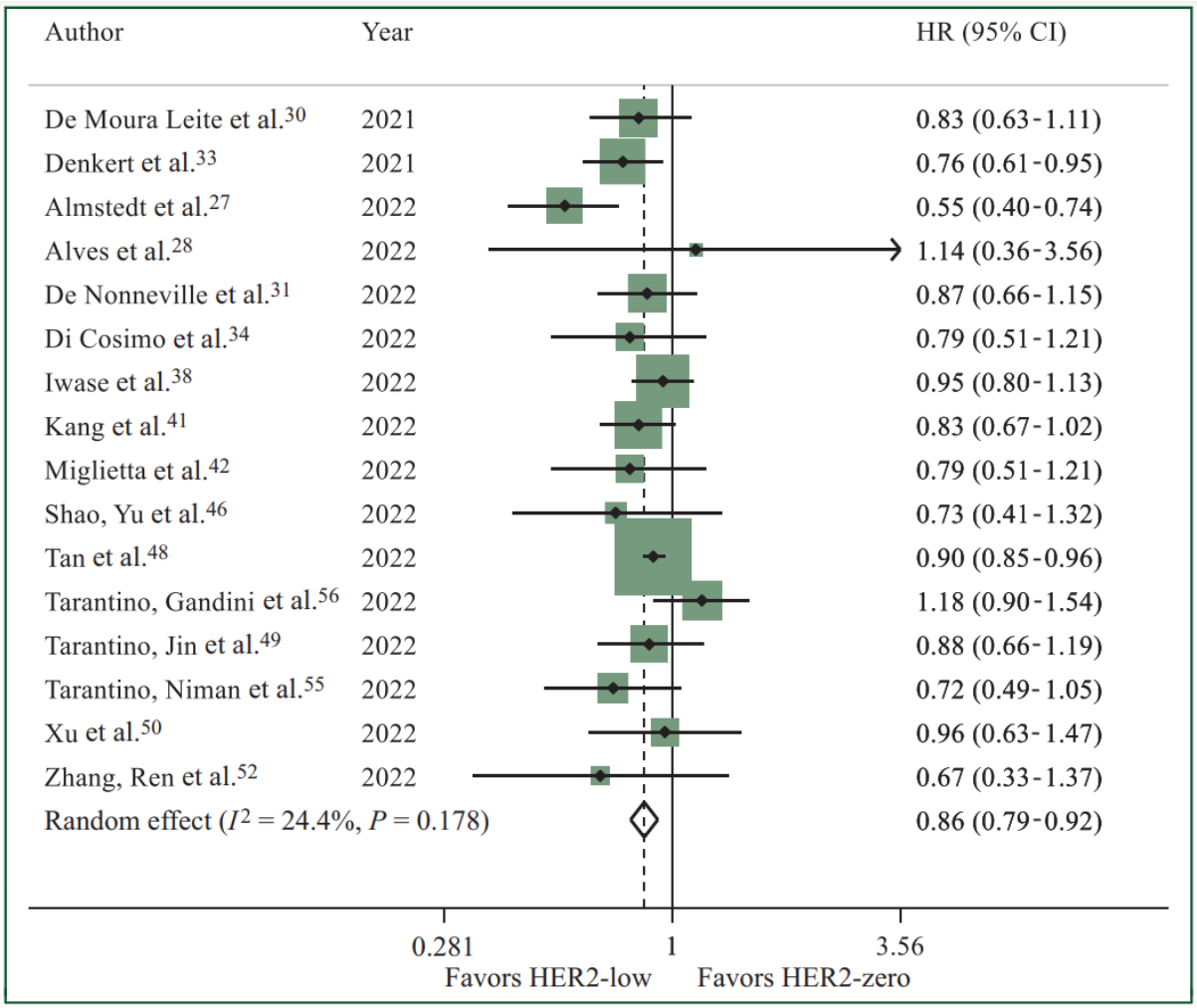


Figure 3. Hazard ratio for disease-free survival of HER2-low breast cancers versus HER2-zero breast cancers in the overall population (the size of the squares is proportional to the weight of each study). CI, confidence interval; HER2, human epidermal growth factor receptor 2. Random effect: P<0.001; Egger's test: P=0.212.

Overall survival

Fourteen studies reported OS data, comparing patients with HER2-low tumours and HER2-zero tumours [27,28,30,33,38,40,41,44,46,48-50,54,55]. Patients with HER2- low tumours had significantly longer OS as compared to those with HER2-zero tumours (HR 0.90, 95% CI 0.85-0.95, P<0.001; $I^{2=}$59.2%; P=0.003) (Figure 4; sensitivity analysis available in the Supplementary Material and Supplementary Table S7). Similar results were observed in the analysis where computed HRs were excluded (data not shown). A potential publication bias was observed (Egger's test: P=0.031). Data about OS in the HoR-positive population were reported in 15 studies [27,30,32,37,38,40,41,43,44,46,48-50,54,55] HER2-low tumours were associated with better OS than HER2- zero tumours (HR 0.94, 95% CI 0.90-0.98, P=0.003; $I^{2=}$47.4%; P=0.021) (Supplementary Figure S5). Consistent results were reported in the sensitivity analysis (Supplementary Table S8). Egger's test P value was <0.001, showing risk of publication bias. OS data in patients with HoR-negative disease were available in 16 studies [27,30,33,35,37,39-41,44,46-50,54,55]. Again, a significant difference in OS was found between the two groups, in favour of HER2-low tumours (HR 0.88, 95% CI 0.82-0.95, P=0.001; $I^{2=}$36.5%; P=0.072; Egger's test: P=0.378) (Supplementary Figure S6). Sensitivity analysis showed the same results after excluding each study one by one (Supplementary Table S9).

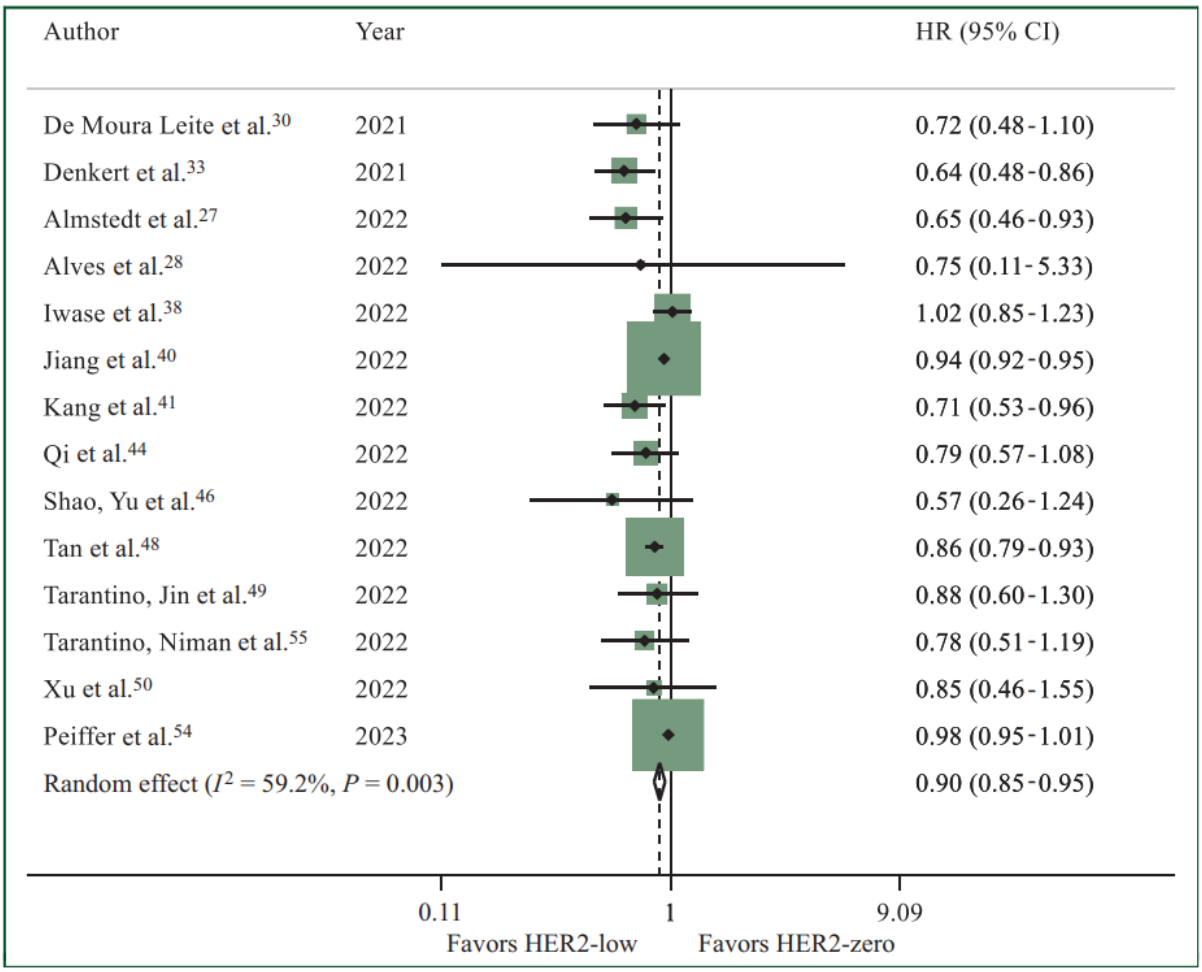


Figure 4. Hazard ratio (HR) for overall survival of HER2-low breast cancers versus HER2-zero breast cancers in the overall population in the early setting (the size of the squares is proportional to the weight of each study). CI, confidence interval; HER2, human epidermal growth factor receptor 2. Random effect: P<0.001; Egger's test: P=0.031.

Metastatic setting

Progression free-survival

Three studies reported data regarding PFS in the overall population [18,19,56]. No significant difference was found in terms of PFS in the first line between HER2-low and HER2-zero tumours (HR 0.99, 95% CI 0.96-1.03, P=0.710; $I^{2=}$0.0%; P=0.541. Egger's test: P=0.300) (Supplementary Figure S7, sensitivity analysis available in the Supplementary Material and

Supplementary Table S10). Five studies reported PFS data for the HoR-positive cohort [15,16,18,19,26]. Consistent with the results obtained for the overall population, there was no significant difference in terms of PFS in the HoR-positive cohort (HR 1.13, 95% CI 0.94-1.35, P=0.192; $I^{2=}$70.8%; P=0.008; Egger's test: P=0.259) (Supplementary Figure S8). Sensitivity analysis demonstrated similar results (Supplementary Table S11). PFS data in the HoR-negative cohort were available in two studies [18,19] and the difference between HER2-low and HER2-zero status was not significant (HR 0.92, 95% CI 0.84-1.02, P=0.103; Egger's test: not computable, sensitivity analysis not carried out) (Supplementary Figure S9).

Overall survival

OS data for the overall population were reported in 10 studies [17-21,23-25,55,56]. A significant difference in terms of OS in favour of patients with HER2-low breast cancer was found in the overall population (HR 0.94, 95% CI 0.89-0.98, P=0.008; I^2=35.3%; P=0.126; Egger's test: P=0.540) (Figure 5; sensitivity analysis available in the Supplementary Material and Supplementary Table S12). Nine studies reported OS data in the HoR-positive cohort [16,18,19,21-23,26,54,55]. As in the overall population, HER2-low status appeared to be associated with better OS when compared to HER2-zero status (HR 0.92, 95% CI 0.87- 0.98, P=0.013; I^2=71.3%, P < 0.001) (Supplementary Figure S10). Data about OS in HoR-negative patients were available in six studies [18-20,23,54,55]. Again, patients affected by HER2-low tumours showed longer OS when compared to patients with HER2-zero tumours (HR 0.91, 95% CI 0.87- 0.95, P<0.001; I^2=0.0%, P=0.981) (Supplementary Figure S11, sensitivity

analysis available in the Supplementary Material and Supplementary Table S14). No significant publication bias was observed in both HoR-positive and HoR-negative subanalyses (Egger's test: P=0.259 and P=0.746, respectively). Risk of bias and publication bias. Eleven studies included were considered to have an overall high RoB [17,20,21,28,32,36,38,40,42,45,50], while 19 studies were classified as having a moderate RoB [15,16,19,22-24,29-31,41,43,44,46,47,52-56], and 12 studies were considered to have a low RoB [18,25-27,33-35,37,39,48,49,51]. A detailed RoB assessment57 for each study is reported in the Supplementary Material.

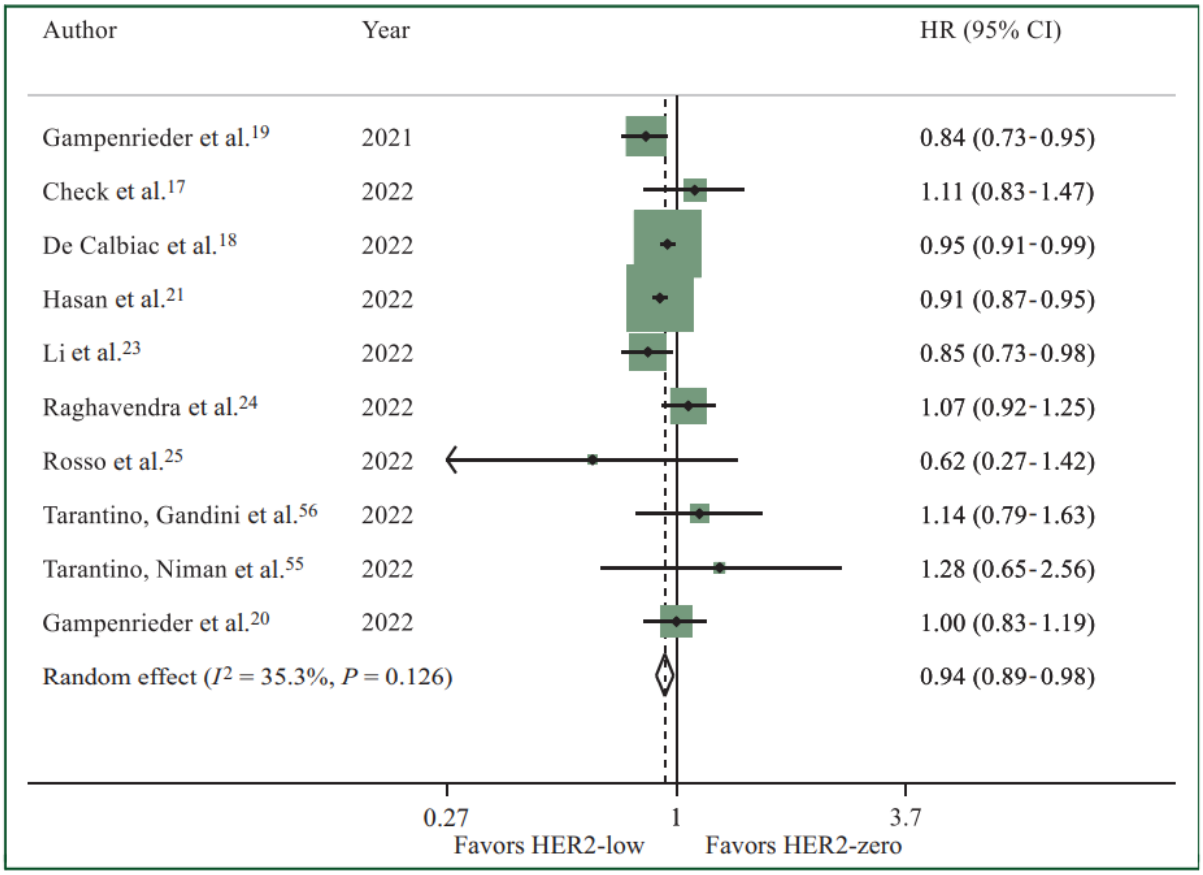


Figure 5. Hazard ratio (HR) for overall survival of HER2-low breast cancers versus HER2-zero breast cancers in the overall population in the metastatic setting (the size of the squares is proportional to the weight of each study). CI, confidence interval; HER2, human epidermal growth factor receptor 2. Random effect: P=0.008; Egger's test: P=0.540.

Discussion

In the past 2 years, HER2-low status has been identified as a new therapeutic target after the impressive results obtained by T-DXd in the phase III DESTINY-Breast04 trial [7]. These data prompted a relevant debate to define whether HER2-low breast cancer could be considered as a new clinicopathological entity or not [10]. This meta-analysis aimed to clarify the prognostic role of HER2-low status. Overall, we included 42 studies with a total of 1 797 175 patients. We observed that HER2-low status appeared to be associated with improved OS regardless of HoR status, both in the advanced and early settings. Moreover, HER2-low status appeared to be associated with a lower rate of pCR as compared to HER2-zero status, in the overall population and HoR-positive subset, but not in triple-negative cases. In the early setting, HER2-low status was associated with longer DFS in the overall population and in patients with HoR-positive disease, while no significant difference was found in the HoR-negative cohort. Among patients with advanced breast cancer, despite the improvement demonstrated in OS, no significant difference was detected in terms of PFS, regardless of HoR status. An explanation for the slightly better prognosis observed in patients with HER2-low tumours might reside in HER2-low tumour biology, apparently strictly associated to HoR status. A lower prevalence of prognostically unfavoured non-luminal tumours in HoR-positive/HER2-low versus HoR-positive/HER2-zero and a direct correlation between HER2-low prevalence and HoR levels have been observed, while no molecular differences have been found in triple-negative HER2-zero versus HER2-low tumours [6,49,58]. A higher prevalence of basal-like tumours in HER2-

zero versus HER2-low breast cancer, driven by the higher prevalence of triple-negative disease in this former IHC category, was also reported [6,58]. Hence, the more favourable prognosis of HER2-low disease might have been influenced by these underlying biological features. At the same time, the relative difference in survival between HER2-low and HER2-zero breast cancer patients is very limited and the statistical significance could be due to the high number of patients included in the analysis and heterogeneity of treatments administered. For these reasons, the better outcomes of HER2-low subgroup may probably translate into limited clinical differences. We also evaluated the association between HER2 status and pCR. HER2-low status appeared to be associated with a lower rate of pCR as compared to HER2-zero status, regardless of HoR status. A substantial heterogeneity was detected in the pCR evaluation among the overall population, while it appeared to be low in the HoR-positive cohort analysis. The results detected in the HoR-positive population are consistent with the data published by Schettini et al [6]. According to their prediction analysis of microarray 50 (PAM50) analysis, only 28.7% of HER2-zero tumours were classified as luminal A [6]. The rate of luminal A subtypes increases when analysing HER2-low IHC 1+ cancers (49%) and HER2-low IHC 2+/ISH not amplified tumours (54.2%). Agostinetto et al. analysed 789 samples with available PAM50 data: among luminal A tumours, the great majority were represented by HER2-low/HoR-positive cancers (54.4%), while 33.7% were HER2-zero/HoR-positive cancers [.58] These data could justify our findings, considering that luminal A breast cancer is characterized by a lower response to chemotherapy and better prognosis than the other subtypes [59,60]. Considering

that the HoR-positive tumours represent the majority of HER2-low breast cancer (from 64% to 93% according to literature) [10], the overall population results could be mostly driven by the HoR-positive cohort. Consistently, in the HoR-negative subgroup analysis, no difference in terms of pCR was detected between HER2-low and HER2-zero breast cancer. According to the studies carried out by Schettini et al. and Agostinetto et al., the majority of triple-negative breast cancers were basal-like through PAM50 analysis, with no significant differences based on HER2 status [6,58]. Coherently, within the basal-like subtype, the rates of HER2-low and HER2-zero tumours were quite similar (41.7% and 40.3%, respectively) [58]. Considering that triple-negative and basal-like breast cancers seem to have a good response to chemotherapy, it is not surprising that no difference was observed in pCR, irrespective of HER2 status. Our data are overall consistent with those published by Denkert et al., who showed that patients with HER2-zero tumours not reaching pCR were those at worst prognosis [33]. As regards the PFS results in the metastatic setting, no differences were found between HER2-low and HER2-zero tumours, in the overall population and regardless of HoR status. In three [15,16,26] out of five studies included in the HoR-positive cohort analysis, the whole cohort was treated in the first line with cyclin-dependent kinase (CDK) 4/6 inhibitors and endocrine therapy. In the study by Gampenrieder et al., 42.7% of patients were treated with this regimen [19]; only 63 out of 15 054 patients received first-line CDK 4/6 inhibitors in the study conducted by de Calbiac et al. [18]. These results are particularly interesting since researchers are actively looking for validated biomarkers to predict the response to CDK 4/6 inhibitors. In the overall population

and HoR-negative cohort, data regarding first-line treatments were scarce. Considering the triple negative subgroup, different regimens could be used as first-line treatment, thus preventing us from drawing solid conclusions. Our meta-analysis has some limitations that should be considered. Firstly, our study is not an individual patient-level data meta-analysis, though it has been shown that individual-level and trial-level pooled analysis results do not diverge significantly, especially for survival data [61-63]. Secondly, almost every study included in our meta-analysis was a retrospective analysis; only one study was prospective [45] and data of two papers were derived from prospective/ retrospective registries [19,26]. Most of the data are derived from national registries, including cancers diagnosed through different decades. A central review of the tumour samples specifically for the considered analysis has been carried out only in two studies [23,39]. Before the discovery of HER2-low status as a therapeutic target, the pathologists were unaware that the distinction of HER2-zero and HER2-low status could guide patient's treatment, so that the historical scores could not be accurate enough to be fully trustable. Moreover, the staining technique and the interpretation (observer-dependent) have been slightly modified over time and significant discordance among pathologists in the evaluation of HER2 status at immunohistochemistry has been demonstrated, especially for HER2 1+ and 2+ categories [6,64]. Furthermore, in the DAISY phase II study, a subgroup of HER2-zero breast tumours partially responded to T-DXd, with a median PFS of 4.2 months [65]. These results strongly suggest that better ways of assessing which patients might benefit from T-DXd are urgently needed. Another issue we had to consider was the

heterogeneity between studies, which was high on four occasions when the pooled estimate was statistically significant (Figures 2 and 4, Supplementary Figures S5 and S10). However, only one study result conflicted with the pooled estimate, and such a merely quantitative heterogeneity did not affect the direction of the pooled estimate. In another case (Supplementary Figure S10), three out of nine studies diverged from the pooled estimate. Yet, they were the least powerful studies and only one reported a statistically significant result, thus not affecting the reliability of the pooled estimate. As regards the metastatic setting, in some studies the HER2 status was assessed on the primary tumour sample [23-25], in others on the sample of the biopsy carried out on the metastatic site if available and on the primary tumour block if the metastatic tissue was not available [16,18,19,22,26,55]. This could be impactful considering the potential significant discordance in terms of HER2 status between primary and metastatic disease, with 44% of breast cancers changing HER2 status from HER2-zero to HER2-low and 22% vice versa [56]. By contrast, the strength of our meta-analysis is the number of patients included, amounting to 1 797 175 subjects. To the best of our knowledge, our study is the largest and most up-to-date meta-analysis assessing the prognostic value of HER2-low status as compared with HER2-zero, both in the early and metastatic settings. Furthermore, we provided a comprehensive analysis of the impact of HER2-low status on different clinical outcomes, in both the advanced and early settings. Finally, we found a specific prognostic implication in terms of OS which is consistent across both settings and all subgroups. In conclusion, HER2-low breast cancer cannot be considered a new biologic entity and its differential prognostic

features in reference to HER2-zero disease are limited and likely driven by HoR status and its underlying biology. Nevertheless, its role as a therapeutic target for novel anti-HER2 ADCs is unquestionable, though probably related only to the presence of some levels of HER2 in the tumour cell membrane. In any case, further investigations are needed to establish the possibility of de-escalating treatment in HER2-low breast cancer due to a potential slightly better prognosis over HER2-zero tumours. Ensuring the proper identification of patients with HER2-low disease has become essential to not deny patients a highly effective treatment with novel targeted agents. To achieve this goal, education and training of pathologists is an urgent need, because they should dismiss the traditional binary distinction of HER2-positive and HER2-negative disease, and accurately and reproducibly report HER2 status according to the scores of the current ASCO/CAP recommendations [4].

Supplementary materials

Search strategies used in each database:

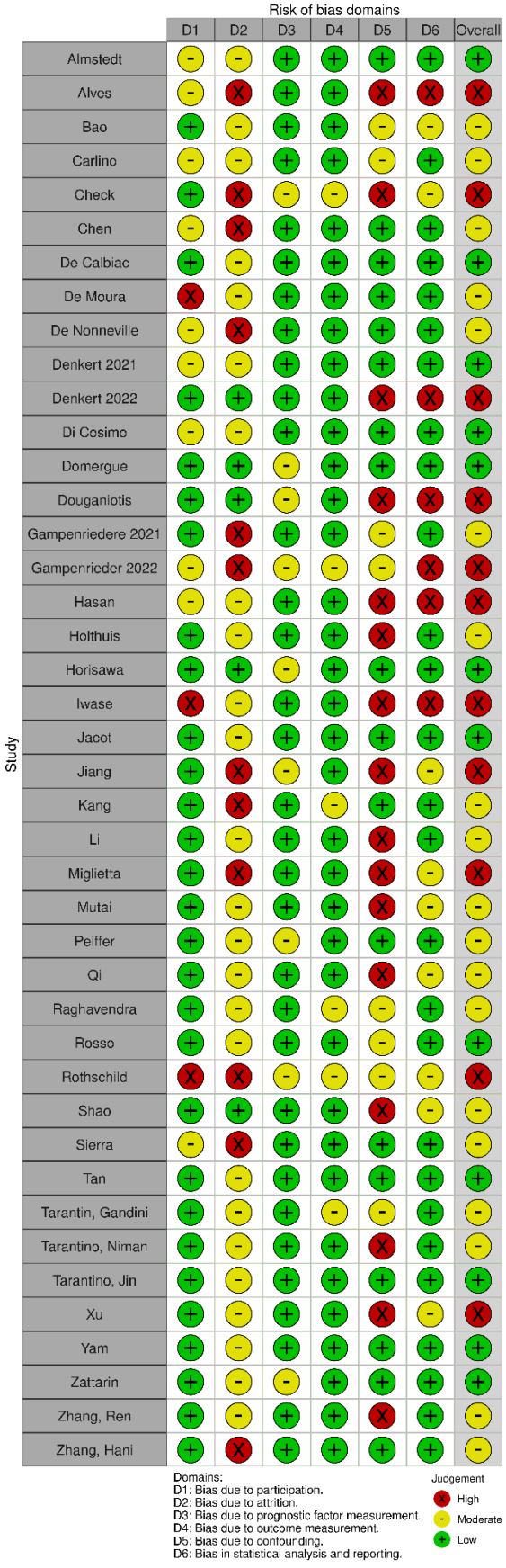
PUBMED

#1	Breast Neoplasms[MeSH Terms] OR breast cancer[Text Word] OR breast neoplasm[Text Word] OR breast tumor[Text Word] OR breast tumour[Text Word] OR breast carcinoma[Text Word] OR cancer breast[Text Word] OR mammary cancer[Text Word] OR mamma cancer[Text Word] OR mammary gland cancer[Text Word] OR breast cancer recurrence[Text Word] OR breast malignancies[Text Word] OR breast malignancy[Text Word] OR breast tumor malignant[Text Word] OR cancer of the breast[Text Word] OR malignant breast neoplasm[Text Word] OR malignant breast tumor[Text Word] OR malignant breast tumour[Text Word] OR malignant neoplasm of the breast[Text Word] OR malignant tumor of the breast[Text Word] OR malignant tumour of the breast[Text Word] OR mammary gland malignancy[Text Word] OR mammary malignancies[Text Word] OR mammary malignancy[Text Word]
#2	((((((HER2-low[Text Word]) OR (HER2 low[Text Word])) OR (ERBB2-low[Text Word])) OR (ERBB2 low[Text Word])) OR (human epidermal growth factor receptor 2 low[Text Word])) OR (low-level HER2[Text Word])) OR (low level HER2[Text Word])
#3	#1 AND #2

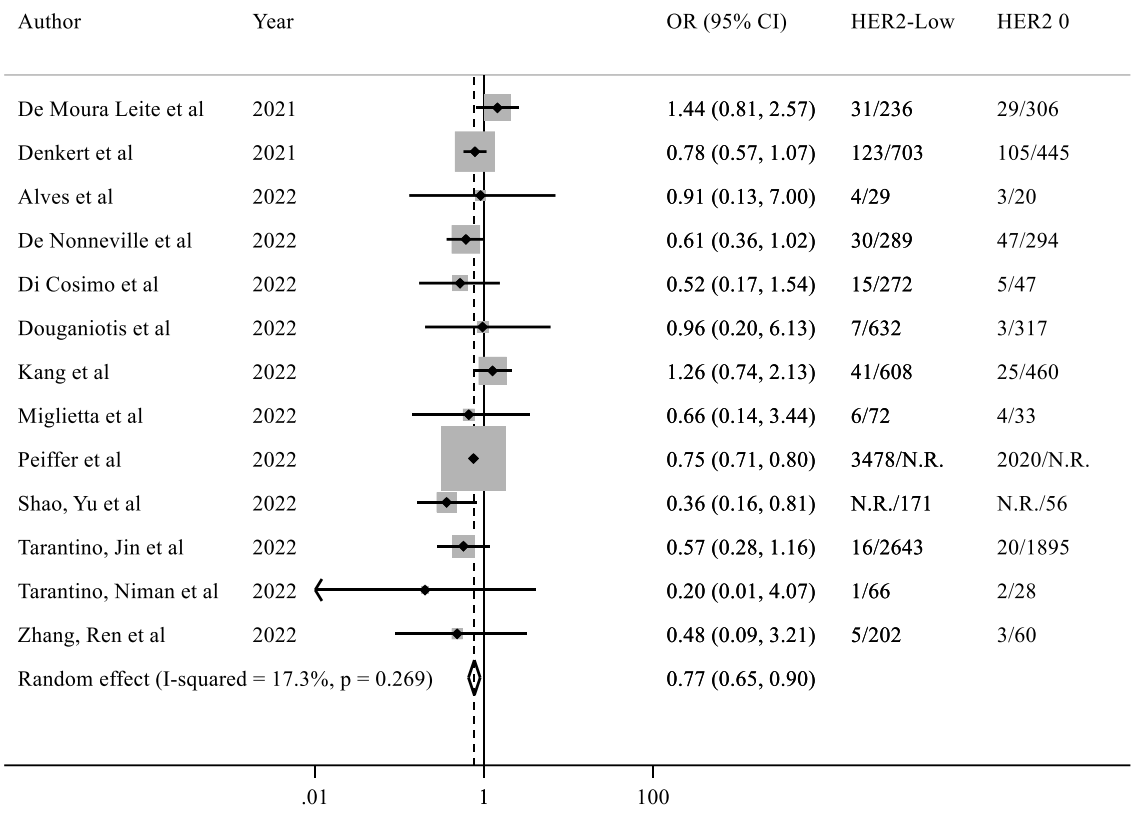
COCHRANE

	Search String
#1	MeSH descriptor: [Breast Neoplasms] 1 tree(s) exploded
#2	(breast cancer):ti,ab,kw OR (breast neoplasm):ti,ab,kw OR (breast tumor):ti,ab,kw OR (breast tumour):ti,ab,kw OR (breast carcinoma):ti,ab,kw OR (breast gland cancer):ti,ab,kw OR (breast gland neoplasm):ti,ab,kw OR (cancer, breast):ti,ab,kw OR (mamma cancer):ti,ab,kw OR (mammary cancer):ti,ab,kw OR (mammary gland cancer):ti,ab,kw OR (breast cancer recurrence):ti,ab,kw
#3	#1 OR #2
#4	(HER2 low):ti,ab,kw OR (ERBB2 Low):ti,ab,kw OR (human epidermal growth factor receptor 2 low):ti,ab,kw OR (low level HER2):ti,ab,kw
#5	#3 AND #4

Detailed RoB assessment:



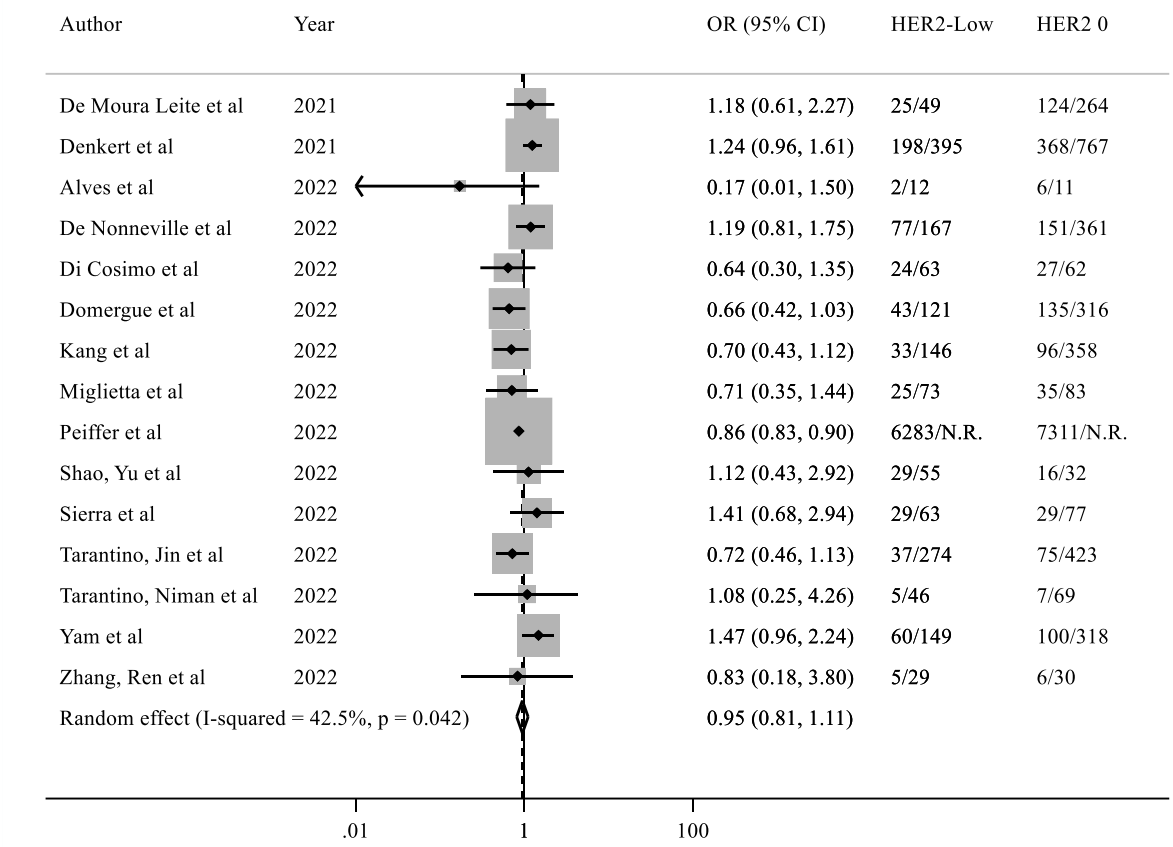
Supplementary Figure S1. Odds ratio for pathological complete response after neoadjuvant chemotherapy of HER2-Low breast cancers vs. HER2-zero breast cancers in hormone receptor-positive population (the size of the squares is proportional to the weight of each study).



Random effect: p=0.001

Egger's test: p=0.804

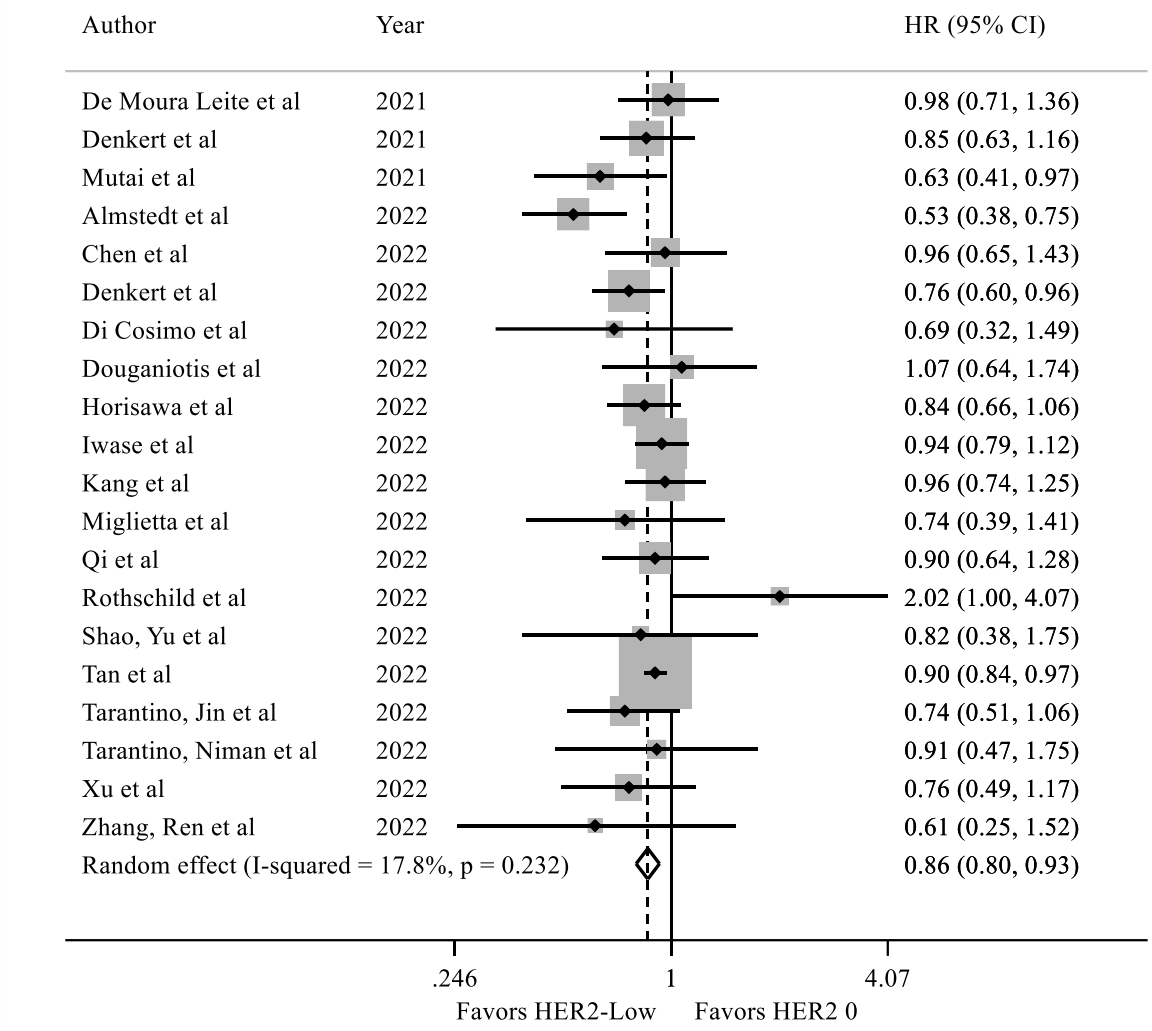
Supplementary Figure S2. Odds ratio for pathological complete response after neoadjuvant chemotherapy of HER2-Low breast cancers vs. HER2-zero breast cancers in hormone receptor-negative population (the size of the squares is proportional to the weight of each study).



Random effect: p=0.497

Egger's test: p=0.513

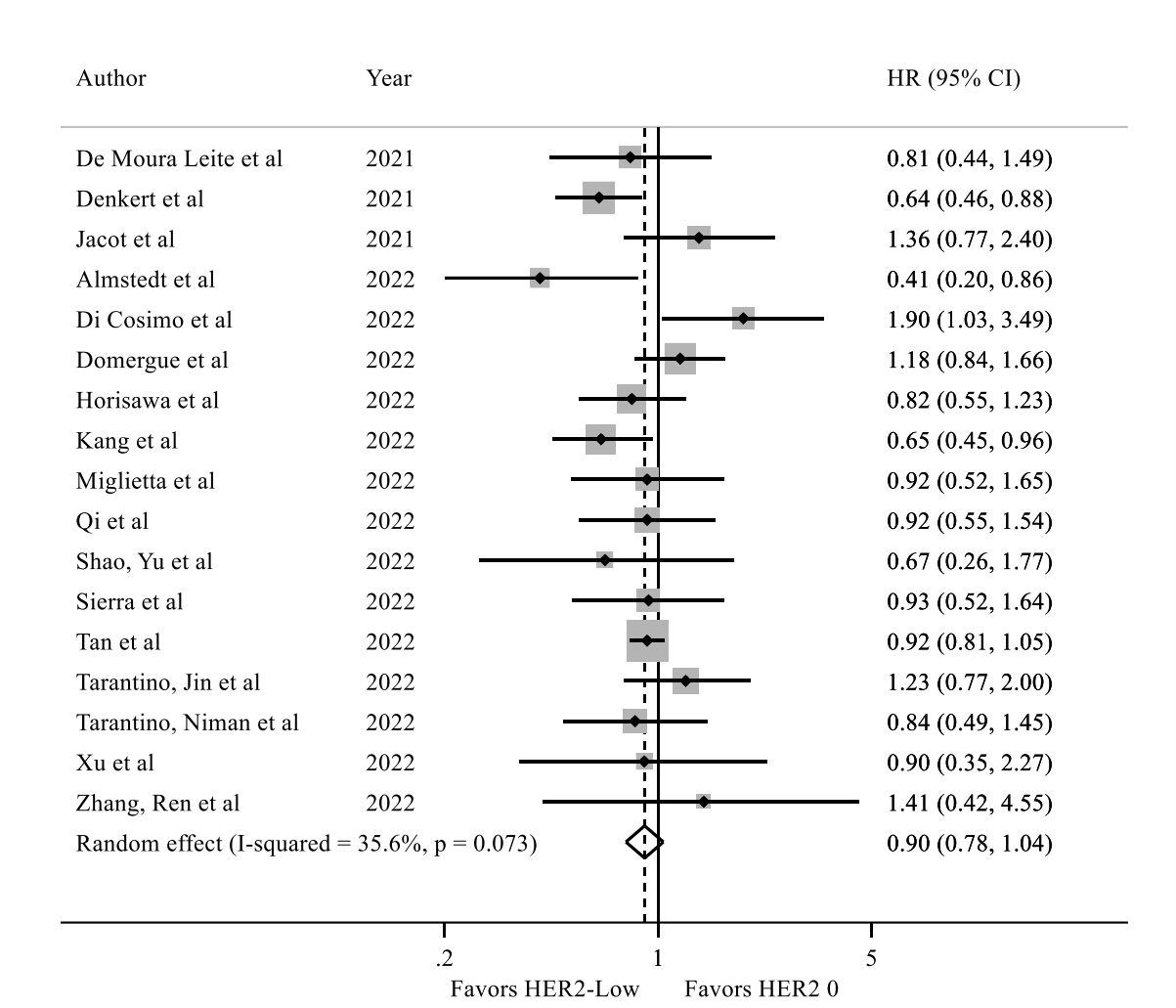
Supplementary Figure S3. Hazard ratio for disease-free survival of HER2-Low breast cancers vs. HER2-zero breast cancers in hormone receptor-positive population (the size of the squares is proportional to the weight of each study).



Random effect: p<0.001

Egger's test: p=0.357

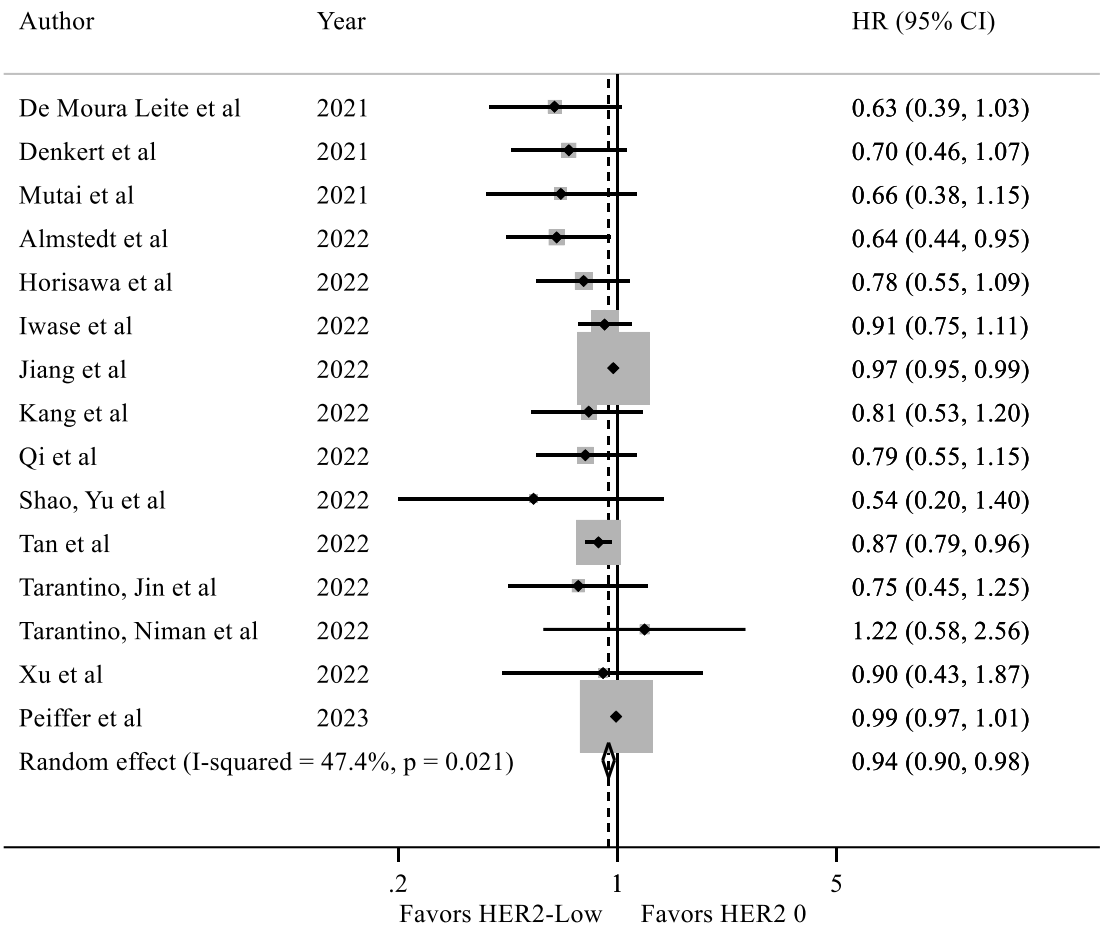
Supplementary Figure S4. Hazard ratio for disease-free survival of HER2-Low breast cancers vs. HER2-zero breast cancers in hormone receptor-negative population (the size of the squares is proportional to the weight of each study).



Random effect: p=0.155

Egger's test: p=0.928

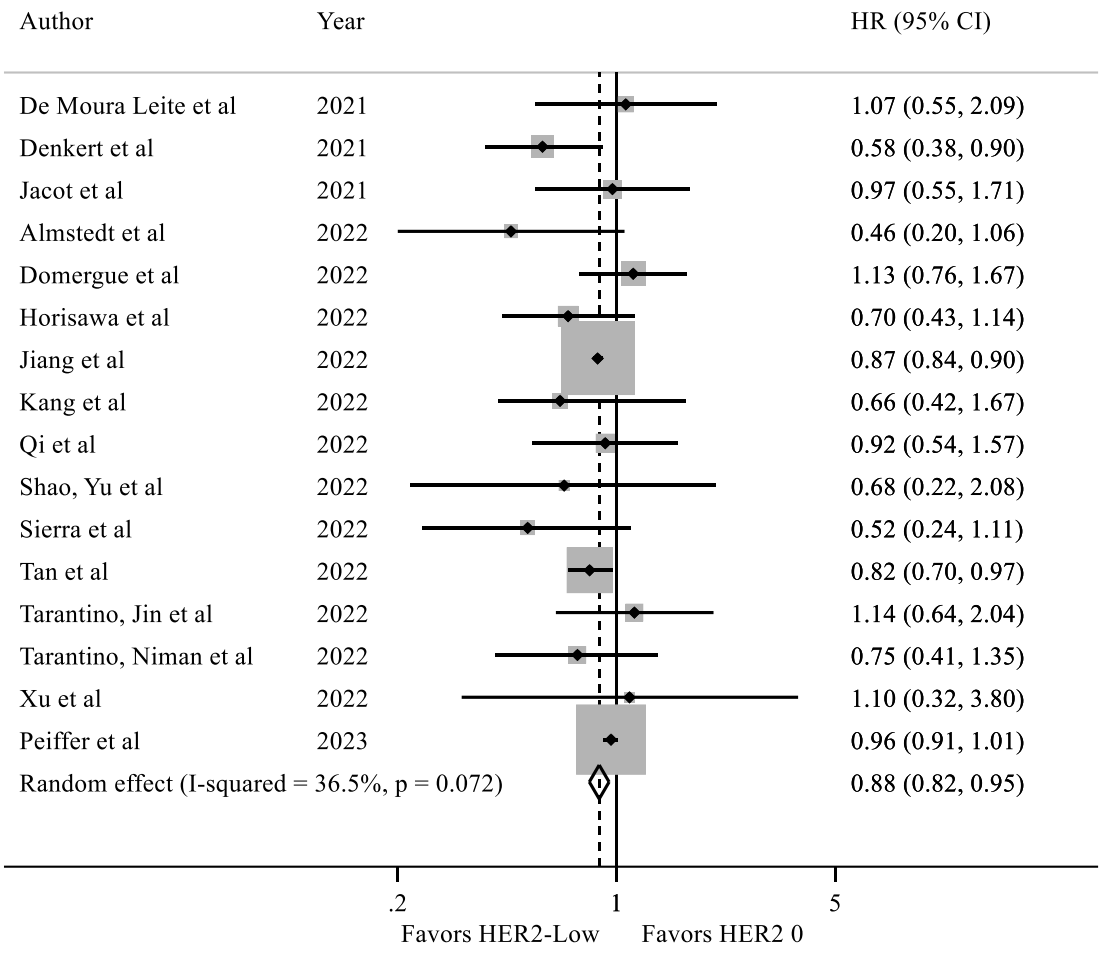
Supplementary Figure S5. Hazard ratio for overall survival of HER2-Low breast cancers vs. HER2-zero breast cancers in hormone receptor-positive population in early setting (the size of the squares is proportional to the weight of each study).



Random effect: p=0.003

Egger's test: p<0.001

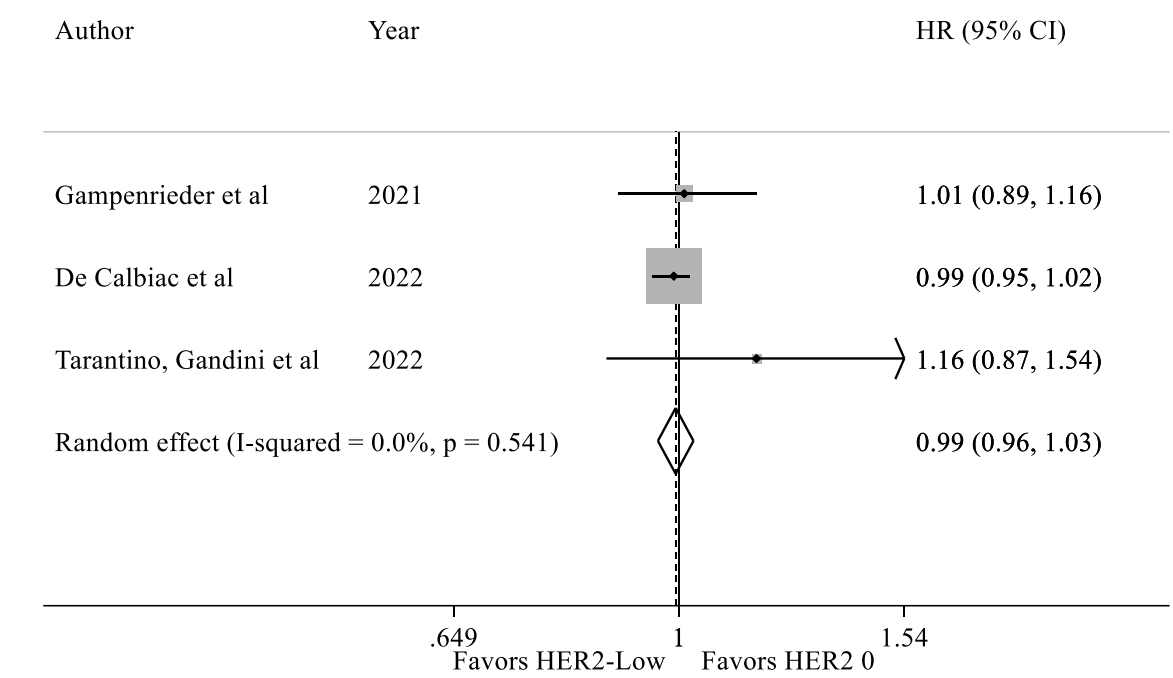
Supplementary Figure S6. Hazard ratio for overall survival of HER2-Low breast cancers vs. HER2-zero breast cancers in hormone receptor-negative population in early setting (the size of the squares is proportional to the weight of each study).



Random effect: p=0.001

Egger's test: p=0.378

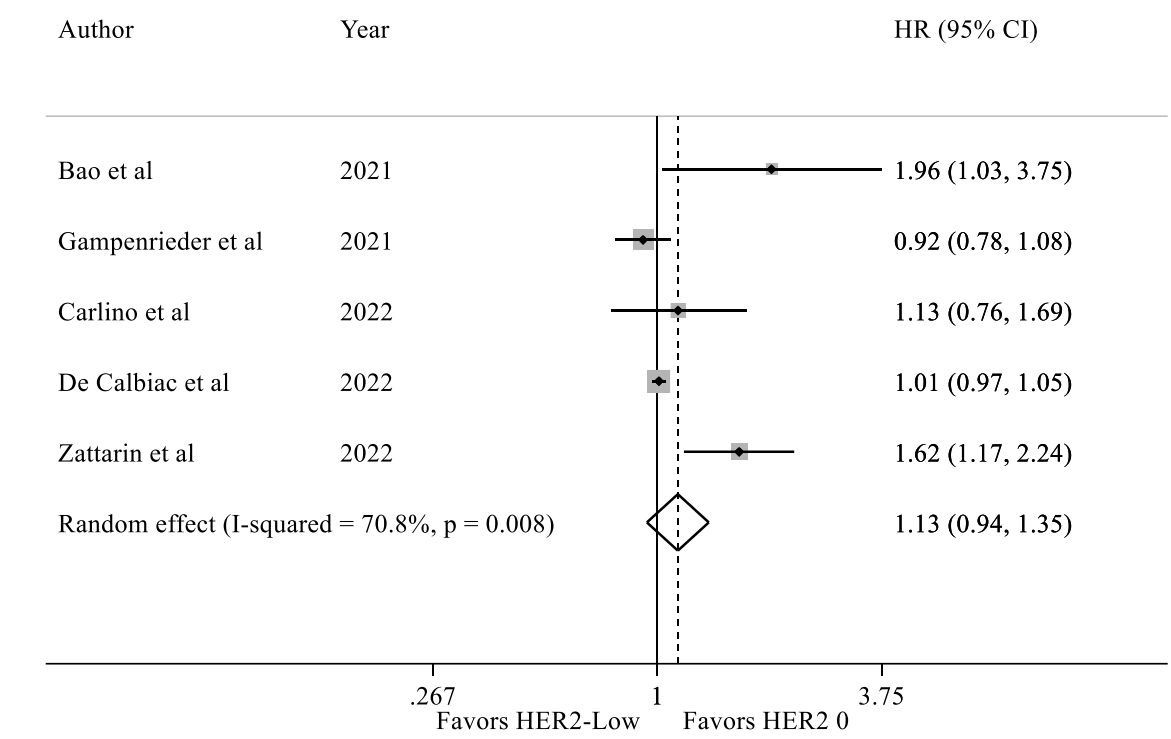
Supplementary Figure S7. Hazard ratio for progression-free survival of HER2-Low breast cancers vs. HER2-zero breast cancers in overall population (the size of the squares is proportional to the weight of each study).



Random effect: p=0.710

Egger's test:p=0.300

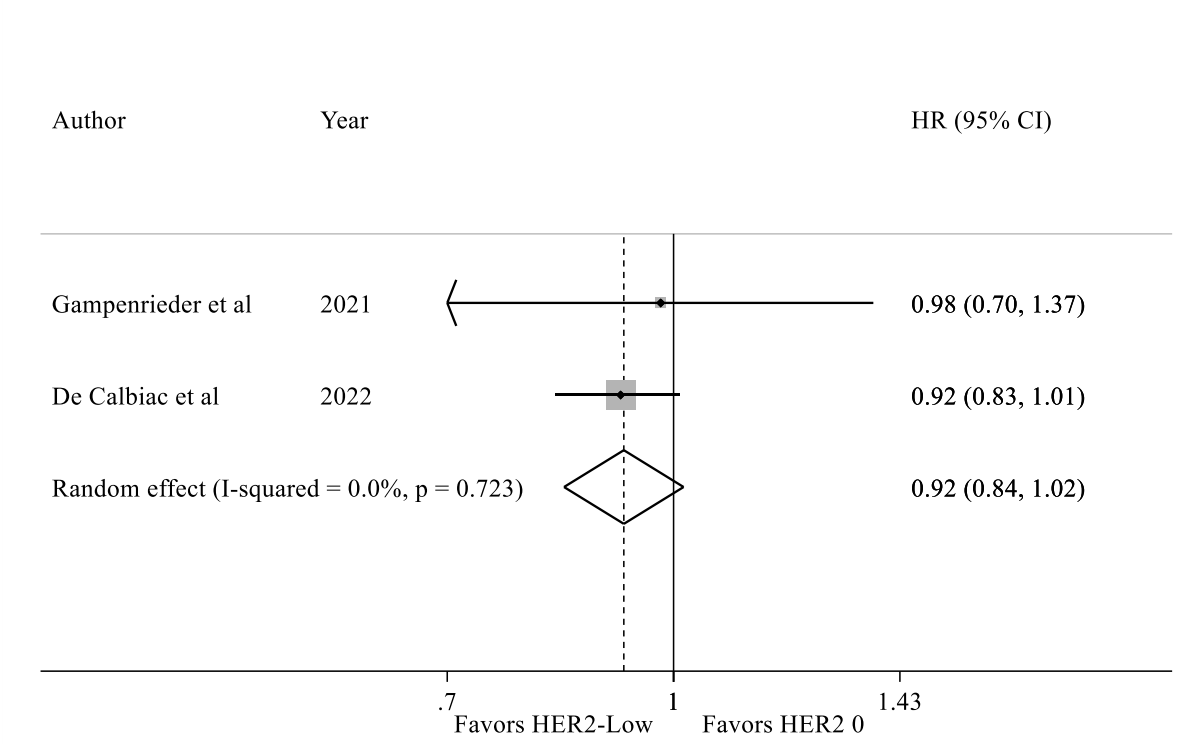
Supplementary Figure S8. Hazard ratio for progression-free survival of HER2-Low breast cancers vs. HER2-zero breast cancers in hormone receptor-positive population (the size of the squares is proportional to the weight of each study).



Random effect: p=0.192

Egger's test: p=0.259

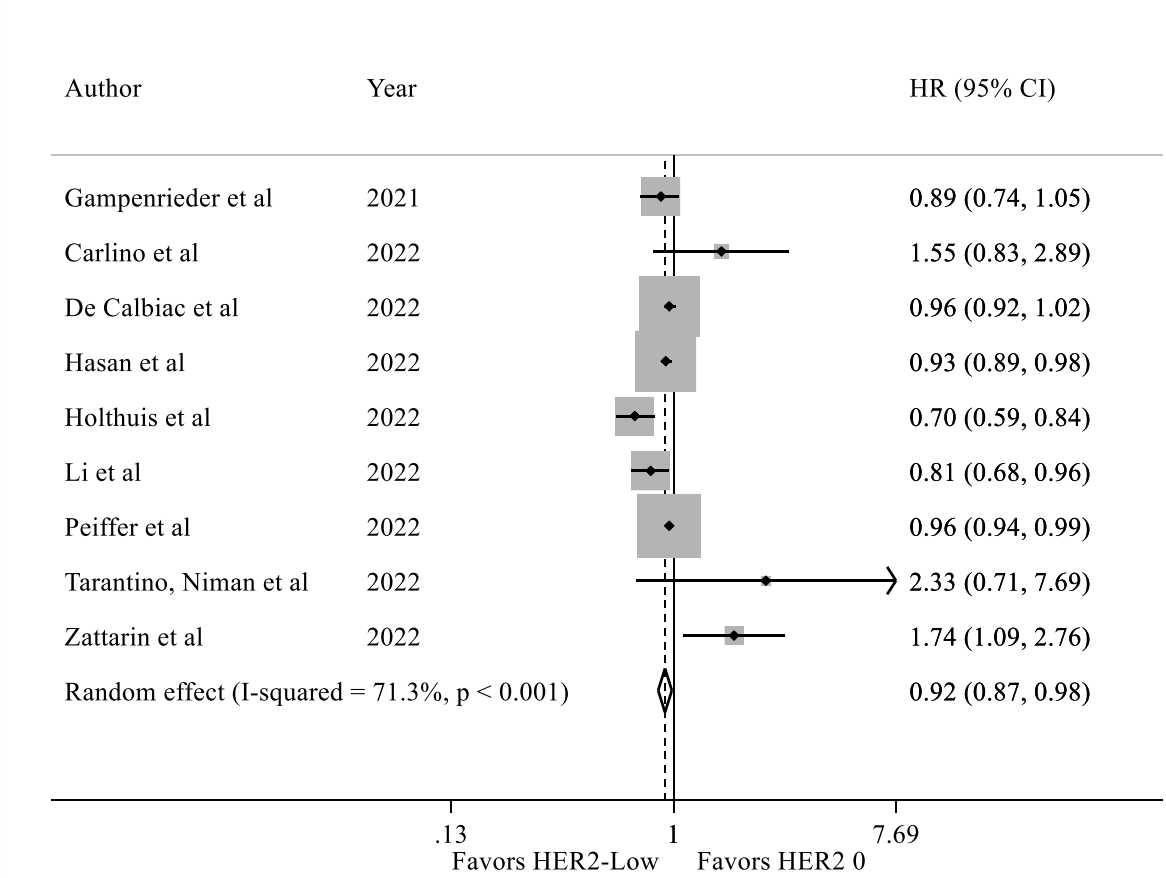
Supplementary Figure S9. Hazard ratio for progression-free survival of HER2-Low breast cancers vs. HER2-zero breast cancers in hormone receptor-negative population (the size of the squares is proportional to the weight of each study).



Random effect: p=0.103

Egger's test: not computable

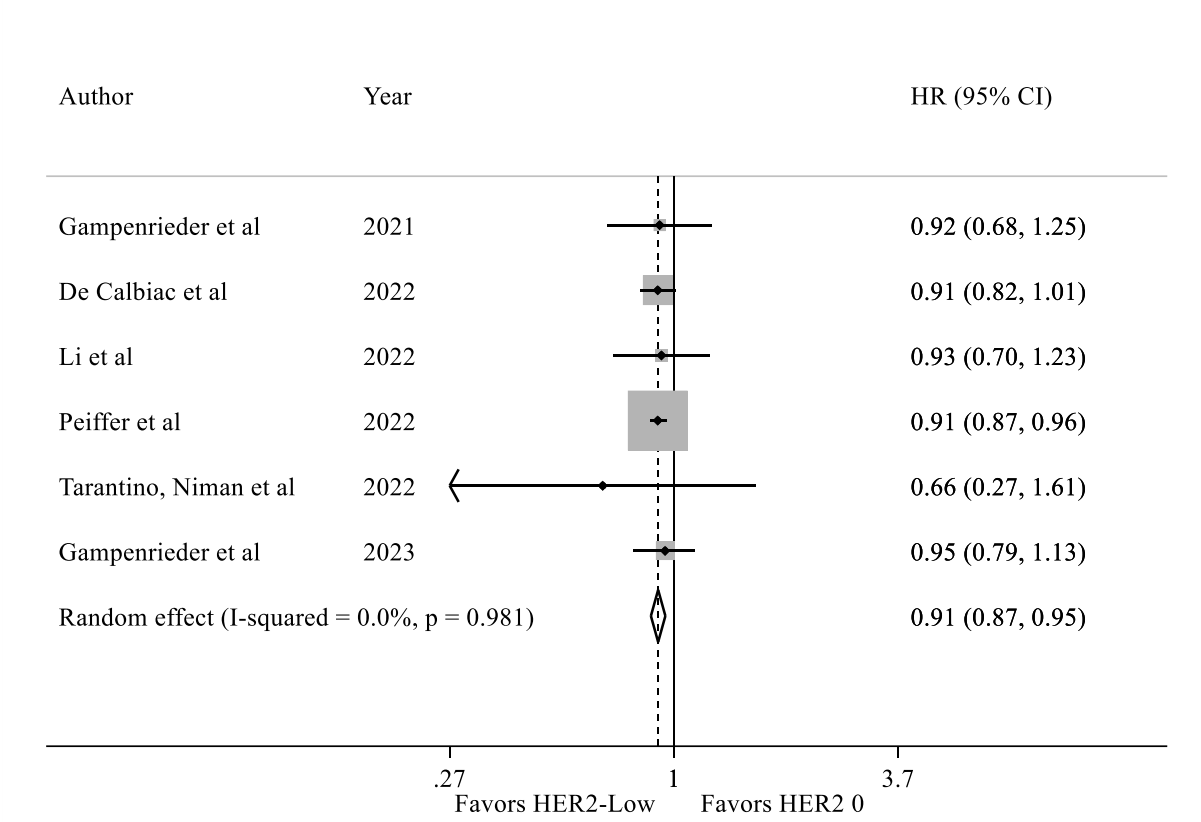
Supplementary Figure S10. Hazard ratio for overall survival of HER2-Low breast cancers vs. HER2-zero breast cancers in hormone receptor-positive population in metastatic setting (the size of the squares is proportional to the weight of each study).



Random effect: p=0.013

Egger's test: p=0.259

Supplementary Figure S11. Hazard ratio for overall survival of HER2-Low breast cancers vs. HER2-zero breast cancers in hormone receptor-negative population in metastatic setting (the size of the squares is proportional to the weight of each study).



Random effect: p<0.001

Egger's test: p=0.746

Supplementary Table T1. Sensitivity analysis, excluding each study one by one, for pathological complete response after neoadjuvant chemotherapy of HER2-Low breast cancers vs. HER2-zero breast cancers in overall population.

Study excluded	Random effect			I-squared (%)	I-sq. P-value
	OR	95% CI	P-value		
De Moura Leite et al 2021	0.74	0.61-0.89	0.002	69.7	<0.001
Denkert et al 2021	0.69	0.56-0.85	<0.001	69.9	<0.001
Alves et al 2022	0.74	0.62-0.88	0.001	70.5	<0.001
De Nonneville et al 2022	0.71	0.58-0.86	0.001	71.3	<0.001
Di Cosimo et al 2022	0.80	0.69-0.93	0.004	56.1	0.007
Iwase et al 2022	0.71	0.59-0.86	<0.001	71.1	<0.001
Kang et al 2022	0.71	0.59-0.86	0.001	71.3	<0.001
Miglietta et al 2022	0.75	0.63-0.90	0.002	69.4	<0.001
Peiffer et al 2022	0.68	0.53-0.87	0.002	69.0	<0.001
Shao, Yu et al 2022	0.72	0.60-0.87	0.001	71.3	<0.001
Tarantino, Jin et al 2022	0.76	0.64-0.91	0.003	66.1	<0.001
Tarantino, Niman et al 2022	0.74	0.62-0.88	0.001	70.9	<0.001
Zhang, Katerji et al 2022	0.76	0.64-0.89	0.001	64.1	0.001
Zhang, Ren et al 2022	0.75	0.63-0.89	0.001	69.1	<0.001

Supplementary Table T2. Sensitivity analysis, excluding each study one by one, for pathological complete response after neoadjuvant chemotherapy of HER2-Low breast cancers vs. HER2-zero breast cancers in hormone-receptor positive population.

Study excluded	Random effect			I-squared (%)	I-sq. P-value
	OR	95% CI	P-value		
De Moura Leite et al 2021	0.75	0.71-0.79	<0.001	0.0	0.564
Denkert et al 2021	0.76	0.61-0.95	0.015	24.0	0.208
Alves et al 2022	0.77	0.64-0.91	0.003	24.0	0.207
De Nonneville et al 2022	0.78	0.65-0.94	0.008	20.7	0.240
Di Cosimo et al 2022	0.77	0.65-0.92	0.004	21.9	0.229
Douganiotis et al 2022	0.77	0.64-0.91	0.003	23.8	0.210
Kang et al 2022	0.75	0.71-0.79	<0.001	0.0	0.457
Miglietta et al 2022	0.77	0.64-0.92	0.003	24.1	0.207
Peiffer et al 2022	0.77	0.60-0.99	0.045	23.0	0.217
Shao, Yu et al 2022	0.76	0.70-0.84	<0.001	2.8	0.417
Tarantino, Jin et al 2022	0.78	0.65-0.93	0.005	21.0	0.237
Tarantino, Niman et al 2022	0.77	0.65-0.91	0.002	20.1	0.246
Zhang, Ren et al 2022	0.77	0.65-0.92	0.003	22.9	0.218

Supplementary Table T3. Sensitivity analysis, excluding each study one by one, for pathological complete response after neoadjuvant chemotherapy of HER2-Low breast cancers vs. HER2-zero breast cancers in hormone-receptor negative population.

Study excluded	Random effect			I-squared (%)	I-sq. P-value
	OR	95% CI	P-value		
De Moura Leite et al 2021	0.94	0.80-1.10	0.434	44.7	0.036
Denkert et al 2021	0.90	0.78-1.04	0.163	23.5	0.199
Alves et al 2022	0.95	0.82-1.11	0.544	42.7	0.045
De Nonneville et al 2022	0.92	0.78-1.09	0.346	40.3	0.059
Di Cosimo et al 2022	0.96	0.82-1.13	0.637	45.1	0.034
Domergue et al 2022	0.98	0.83-1.15	0.789	43.1	0.043
Kang et al 2022	0.97	0.82-1.15	0.725	44.7	0.036
Miglietta et al 2022	0.96	0.81-1.13	0.617	45.9	0.031
Peiffer et al 2022	0.97	0.80-1.17	0.742	33.2	0.110
Shao, Yu et al 2022	0.94	0.80-1.11	0.478	46.0	0.030
Sierra et al 2022	0.93	0.80-1.09	0.387	42.6	0.046
Tarantino, Jin et al 2022	0.97	0.82-1.15	0.726	45.0	0.035
Tarantino, Niman et al 2022	0.95	0.80-1.11	0.496	46.4	0.029
Yam et al 2022	0.91	0.79-1.05	0.200	29.4	0.142
Zhang, Ren et al 2022	0.95	0.81-1.11	0.520	46.6	0.028

Supplementary Table T4. Sensitivity analysis, excluding each study one by one, for disease-free survival of HER2-Low breast cancers vs. HER2-zero breast cancers in overall population.

Study excluded	Random effect			I-squared (%)	I-sq. P-value
	HR	95% CI	P-value		
De Moura Leite et al 2021	0.85	0.79-0.93	<0.001	28.8	0.141
Denkert et al 2021	0.87	0.80-0.94	<0.001	22.6	0.202
Almstedt et al 2022	0.89	0.85-0.93	<0.001	0.0	0.711
Alves et al 2022	0.85	0.79-0.92	<0.001	28.7	0.142
De Nonneville et al 2022	0.85	0.78-0.92	<0.001	29.4	0.135
Di Cosimo et al 2022	0.86	0.79-0.93	<0.001	28.6	0.143
Iwase et al 2022	0.84	0.77-0.92	<0.001	26.3	0.165
Kang et al 2022	0.86	0.79-0.93	<0.001	28.3	0.146
Miglietta et al 2022	0.86	0.79-0.93	<0.001	28.6	0.143
Shao, Yu et al 2022	0.86	0.79-0.93	<0.001	28.0	0.149
Tan et al 2022	0.84	0.76-0.92	<0.001	23.7	0.191
Tarantino, Gandini et al 2022	0.86	0.80-0.91	<0.001	7.1	0.373
Tarantino, Jin et al 2022	0.85	0.78-0.92	<0.001	29.4	0.135
Tarantino, Niman et al 2022	0.86	0.80-0.93	<0.001	25.4	0.174
Xu et al 2022	0.85	0.78-0.92	<0.001	28.8	0.141
Zhang, Ren et al 2022	0.86	0.79-0.93	<0.001	27.4	0.155

Supplementary Table T5. Sensitivity analysis, excluding each study one by one, for disease-free survival of HER2-Low breast cancers vs. HER2-zero breast cancers in hormone receptor-positive population.

Study excluded	Random effect			I-squared (%)	I-sq. P-value
	HR	95% CI	P-value		
De Moura Leite et al 2021	0.85	0.79-0.92	<0.001	20.5	0.205
Denkert et al 2021	0.86	0.79-0.93	<0.001	22.0	0.188
Mutai et al 2021	0.87	0.81-0.93	<0.001	13.5	0.289
Almstedt et al 2022	0.89	0.84-0.94	<0.001	0.0	0.697
Chen et al 2022	0.85	0.79-0.92	<0.001	21.4	0.194
Denkert et al 2022	0.87	0.80-0.94	<0.001	16.8	0.249
Di Cosimo et al 2022	0.86	0.79-0.93	<0.001	20.8	0.201
Douganiotis et al 2022	0.85	0.79-0.92	<0.001	20.0	0.211
Horisawa et al 2022	0.86	0.79-0.93	<0.001	21.7	0.191
Iwase et al 2022	0.85	0.78-0.92	<0.001	19.8	0.213
Kang et al 2022	0.85	0.78-0.92	<0.001	20.5	0.205
Miglietta et al 2022	0.86	0.79-0.93	<0.001	21.2	0.197
Qi et al 2022	0.85	0.79-0.93	<0.001	22.0	0.187
Rothschild et al 2022	0.87	0.83-0.92	<0.001	0.0	0.479
Shao, Yu et al 2022	0.86	0.79-0.93	<0.001	22.0	0.187
Tan et al 2022	0.84	0.77-0.92	<0.001	18.0	0.234
Tarantino, Jin et al 2022	0.86	0.80-0.93	<0.001	19.2	0.220
Tarantino, Niman et al 2022	0.86	0.79-0.93	<0.001	22.1	0.187
Xu et al 2022	0.86	0.79-0.93	<0.001	20.7	0.203
Zhang, Ren et al 2022	0.86	0.80-0.93	<0.001	20.0	0.211

Supplementary Table T6. Sensitivity analysis, excluding each study one by one, for disease-free survival of HER2-Low breast cancers vs. HER2-zero breast cancers in hormone receptor-negative population.

Study excluded	Random effect			I-squared (%)	I-sq. P-value
	HR	95% CI	P-value		
De Moura Leite et al 2021	0.91	0.78-1.05	0.201	39.3	0.054
Denkert et al 2021	0.94	0.82-1.07	0.350	25.4	0.167
Jacot et al 2021	0.88	0.77-1.02	0.091	34.2	0.089
Almstedt et al 2022	0.92	0.81-1.05	0.222	25.9	0.163
Di Cosimo et al 2022	0.88	0.77-0.99	0.038	21.0	0.214
Domergue et al 2022	0.88	0.76-1.02	0.081	32.7	0.101
Horisawa et al 2022	0.91	0.78-1.06	0.229	39.0	0.056
Kang et al 2022	0.93	0.81-1.07	0.311	31.0	0.115
Miglietta et al 2022	0.90	0.78-1.05	0.178	39.6	0.052
Qi et al 2022	0.90	0.78-1.05	0.180	39.6	0.052
Shao, Yu et al 2022	0.91	0.79-1.05	0.195	38.6	0.058
Sierra et al 2022	0.90	0.78-1.05	0.175	39.6	0.052
Tan et al 2022	0.90	0.76-1.07	0.243	39.2	0.054
Tarantino, Jin et al 2022	0.88	0.76-1.02	0.098	35.2	0.081
Tarantino, Niman et al 2022	0.91	0.78-1.05	0.200	39.4	0.053

Xu et al 2022	0.90	0.78-1.05	0.173	39.6	0.052
Zhang, Ren et al 2022	0.90	0.78-1.04	0.141	38.2	0.060

Supplementary Table T7. Sensitivity analysis, excluding each study one by one, for overall survival in early setting of HER2-Low breast cancers vs. HER2-zero breast cancers in overall population.

Study excluded	**Random effect**			**I-squared (%)**	**I-sq. P-value**
	HR	**95% CI**	**P-value**		
De Moura Leite et al 2021	0.90	0.86-0.95	<0.001	60.3	0.003
Denkert et al 2021	0.92	0.87-0.96	<0.001	52.1	0.015
Almstedt et al 2022	0.91	0.87-0.96	<0.001	56.5	0.006
Alves et al 2022	0.90	0.85-0.95	<0.001	62.3	0.001
Iwase et al 2022	0.89	0.84-0.94	<0.001	61.5	0.002
Jiang et al 2022	0.84	0.76-0.92	<0.001	61.6	0.002
Kang et al 2022	0.91	0.86-0.96	<0.001	57.7	0.005
Qi et al 2022	0.90	0.86-0.95	<0.001	60.9	0.002
Shao, Yu et al 2022	0.90	0.86-0.95	<0.001	60.4	0.003
Tan et al 2022	0.91	0.86-0.97	0.002	55.2	0.008
Tarantino, Jin et al 2022	0.90	0.85-0.95	<0.001	62.2	0.002
Tarantino, Niman et al 2022	0.90	0.85-0.95	<0.001	61.4	0.002
Xu et al 2022	0.90	0.85-0.95	<0.001	62.2	0.002
Peiffer et al 2022	0.84	0.77-0.92	<0.001	50.5	0.019

Supplementary Table T8. Sensitivity analysis, excluding each study one by one, for overall survival in early setting of HER2-Low breast cancers vs. HER2-zero breast cancers in hormone receptor-positive population.

Study excluded	**Random effect**			**I-squared (%)**	**I-sq. P-value**
	HR	**95% CI**	**P-value**		
De Moura Leite et al 2021	0.94	0.91-0.98	0.006	44.8	0.036
Denkert et al 2021	0.94	0.90-0.98	0.006	46.4	0.029
Mutai et al 2021	0.94	0.90-0.98	0.005	47.4	0.025
Almstedt et al 2022	0.95	0.91-0.99	0.007	41.0	0.055
Horisawa et al 2022	0.94	0.90-0.98	0.005	48.0	0.023
Iwase et al 2022	0.94	0.89-0.98	0.005	50.3	0.016
Jiang et al 2022	0.85	0.77-0.94	0.001	50.5	0.016
Kang et al 2022	0.94	0.90-0.98	0.004	49.7	0.018
Qi et al 2022	0.94	0.90-0.98	0.005	48.8	0.020
Shao, Yu et al 2022	0.94	0.90-0.98	0.004	48.5	0.022
Tan et al 2022	0.95	0.92-0.99	0.027	39.0	0.067
Tarantino, Jin et al 2022	0.94	0.90-0.98	0.004	49.3	0.019
Tarantino, Niman et al 2022	0.93	0.89-0.98	0.003	50.5	0.016
Xu et al 2022	0.93	0.89-0.98	0.003	51.1	0.014
Peiffer et al 2022	0.85	0.78-0.93	0.001	41.1	0.054

Supplementary Table T9. Sensitivity analysis, excluding each study one by one, for overall survival in early setting of HER2-Low breast cancers vs. HER2-zero breast cancers in hormone receptor-negative population.

Study excluded	Random effect			I-squared (%)	I-sq. P-value
	HR	95% CI	P-value		
De Moura Leite et al 2021	0.88	0.81-0.94	0.001	40.0	0.055
Denkert et al 2021	0.89	0.84-0.95	0.001	29.2	0.137
Jacot et al 2021	0.88	0.81-0.95	0.001	40.5	0.052
Almstedt et al 2022	0.89	0.83-0.95	0.001	34.0	0.097
Domergue et al 2022	0.87	0.81-0.94	<0.001	37.0	0.074
Horisawa et al 2022	0.88	0.82-0.95	0.001	38.3	0.066
Jiang et al 2022	0.87	0.78-0.97	0.011	22.1	0.208
Kang et al 2022	0.88	0.82-0.95	0.001	38.8	0.062
Qi et al 2022	0.88	0.81-0.95	0.001	40.7	0.051
Shao, Yu et al 2022	0.88	0.82-0.95	0.001	40.2	0.054
Sierra et al 2022	0.88	0.82-0.95	0.001	35.5	0.085
Tan et al 2022	0.89	0.82-0.96	0.004	38.0	0.067
Tarantino, Jin et al 2022	0.88	0.81-0.94	<0.001	38.9	0.061
Tarantino, Niman et al 2022	0.88	0.82-0.95	0.001	39.9	0.056
Xu et al 2022	0.88	0.81-0.95	0.001	40.5	0.052
Peiffer et al 2022	0.87	0.84-0.89	<0.001	0.0	0.541

Supplementary Table T10. Sensitivity analysis, excluding each study one by one, for progression-free survival of HER2-Low breast cancers vs. HER2-zero breast cancers in overall population.

Study excluded	Random effect			I-squared (%)	I-sq. P-value
	HR	95% CI	P-value		
Gampenrieder et al 2021	1.00	0.92-1.09	0.941	14.2	0.280
De Calbiac et al 2022	1.03	0.92-1.17	0.574	0.0	0.389
Tarantino, Gandini et al 2022	0.99	0.96-1.03	0.619	0.0	0.775

Supplementary Table T11. Sensitivity analysis, excluding each study one by one, for progression-free survival of HER2-Low breast cancers vs. HER2-zero breast cancers in hormone-receptor positive population.

Study excluded	Random effect			I-squared (%)	I-sq. P-value
	HR	95% CI	P-value		
Bao et al 2021	1.07	0.91-1.27	0.395	69.0	0.021
Gampenrieder et al 2021	1.29	0.95-1.76	0.108	75.4	0.007
Carlino et al 2022	1.14	0.92-1.41	0.219	77.6	0.004
De Calbiac et al 2022	1.27	0.89-1.81	0.181	77.0	0.005
Zattarin et al 2022	1.02	0.89-1.16	0.799	46.5	0.133

Supplementary Table T12. Sensitivity analysis, excluding each study one by one, for overall survival in metastatic setting of HER2-Low breast cancers vs. HER2-zero breast cancers in overall population.

Study excluded	Random effect			I-squared (%)	I-sq. P-value
	HR	95% CI	P-value		
Gampenrieder et al 2021	0.95	0.90-0.99	0.022	29.8	0.180
Check et al 2022	0.93	0.89-0.98	0.004	35.7	0.132
De Calbiac et al 2022	0.94	0.87-1.01	0.074	35.9	0.131
Hasan et al 2022	0.95	0.89-1.02	0.143	33.3	0.151
Li et al 2022	0.94	0.90-0.99	0.026	35.2	0.137
Raghavendra et al 2022	0.92	0.88-0.97	0.001	25.1	0.221
Rosso et al 2022	0.94	0.89-0.98	0.010	38.3	0.113
Tarantino, Gandini et al 2022	0.93	0.89-0.98	0.005	37.0	0.122
Tarantino, Niman et al 2022	0.93	0.89-0.98	0.008	38.8	0.109
Gampenrieder et al 2023	0.93	0.88-0.98	0.009	39.8	0.102

Supplementary Table T13. Sensitivity analysis, excluding each study one by one, for overall survival in metastatic setting of HER2-Low breast cancers vs. HER2-zero breast cancers in hormone receptor-positive population.

Study excluded	Random effect			I-squared (%)	I-sq. P-value
	HR	95% CI	P-value		
Gampenrieder et al 2021	0.93	0.87-0.99	0.026	74.4	<0.001
Carlino et al 2022	0.92	0.86-0.98	0.008	72.6	0.001
De Calbiac et al 2022	0.91	0.84-0.99	0.034	74.7	<0.001
Hasan et al 2022	0.92	0.84-1.01	0.059	74.1	<0.001
Holthuis et al 2022	0.95	0.90-0.99	0.033	57.1	0.022
Li et al 2022	0.94	0.88-0.99	0.040	71.5	0.001
Peiffer et al 2022	0.91	0.83-1.01	0.065	73.0	0.001
Tarantino, Niman et al 2022	0.92	0.87-0.98	0.009	72.8	0.001
Zattarin et al 2022	0.92	0.87-0.97	0.003	67.2	0.003

Supplementary Table T14. Sensitivity analysis, excluding each study one by one, for overall survival in metastatic setting of HER2-Low breast cancers vs. HER2-zero breast cancers in hormone receptor-negative population.

Study excluded	Random effect			I-squared (%)	I-sq. P-value
	HR	95% CI	P-value		
Gampenrieder et al 2021	0.91	0.87-0.95	<0.001	0.0	0.947
De Calbiac et al 2022	0.91	0.87-0.96	<0.001	0.0	0.947
Li et al 2022	0.91	0.87-0.95	<0.001	0.0	0.949
Peiffer et al 2022	0.92	0.85-0.99	0.042	0.0	0.951
Tarantino, Niman et al 2022	0.91	0.87-0.95	<0.001	0.0	0.994
Gampenrieder et al 2023	0.91	0.87-0.95	<0.001	0.0	0.971

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